The Effect of Vitamin C on the Flavivirus

Curtis T. Hibbert[1]

[1]University of Science, Arts and Technology

Abstract

With the exception of the *Fenton Reaction*, ascorbic acid (vitamin c) has been known to be an effective antioxidant that inhibits the oxidative effects of molecules in the human body. This antioxidant has been known to reduce the production of free radicals and limit pro-oxidative induced chemical reactions that cause both cell damage and oxidative stress. In relation to the human body and the unimpeded production of free radicals (oxidative stress), recent studies have shown that the Flavivirus genus, which includes the West Nile virus, Dengue virus, Tick-Borne Encephalitis virus, Yellow Fever virus, and Zika virus has shown promising results of infection reversal after vitamin c supplementation. The biological mechanism behind such reversal is believed to be contingent upon immunity support, and most recently, oxidative stress reduction during infection. The idea behind oxidative stress reduction revolves around the belief that vitamin c can temporally control genome RNA capping and replication, by inhibition and donating elections, causing an antioxidant effect. These findings suggest that vitamin c has an effect on the Flavivirus *in vivo* with regards to immunity support and replication inhibition.

Keywords: Flavivirus, Zika virus, Dengue virus, West Nile virus, Vitamin C, Antioxidant, Oxidative Stress

Conflict of Interest: The authors have no conflicts of interest to disclose.

The Effect of Vitamin C on the Flavivirus

Review Article

The Flavivirus genus is derived from the family of Flaviviridae, which includes positive, single-stranded, enveloped RNA viruses (7, 10). This genus, which also is commonly identified as arboviruses, includes the West Nile virus, Dengue virus, Tick-Borne Encephalitis virus, Yellow Fever virus, and the Zika virus. The Flavivirus is notorious for being the source of infections worldwide that leads to both high morbidity and mortality rates (7, 14, 21). Moreover, with the recent spread of the Zika virus, the effects of this epidemic has been believed to have caused hundreds of cases of birth defects and microcephaly. The universal method of treatment for some of these viruses, such as the Zika virus is still unclear, making it imperative that we gain a better understanding of how these viruses survive in the human body and can be treated.

Treatment of virally infected cells with antioxidants, such as vitamin c has been a known method of treatment for various forms of infection for many years; however, such treatment hasn't been considered to be a mainstream approach (1, 3). Nevertheless, vitamin c has been scientifically known to relieve oxidative stress in patients for many years; which consequently improve disease outcomes, especially outcomes stemming from viral infections, such as the Flavivirus (14, 21). In addition, more research is needed to determine the true effect of vitamin c on the Flavivirus. In this review, we will examine ascorbic acid, the Flavivirus genus and the effect of vitamin c on the Flavivirus.

Vitamin C

Vitamin C or ascorbic acid is considered to be an essential nutrient for the human body (1, 3). The biological role of vitamin c is based on the fundamental idea of it acting as a reducing agent, and targeting free radicals in the human body by donating electrons to various enzymatic and non-enzymatic reactions (3). In addition to ascorbic acid's antioxidant functions, vitamin c plays an important role in the immune system; this role ranges from interferon production support to the inhibition of various forms of T-lymphocyte expiration (12).

The positive effect of vitamin c is often attributed to its role in the human body as an antioxidant; however, vitamin c can perform various additional physiological and biological roles within the human body (2, 3, 9, 22). These roles include the support of the immune system to the inhibition of pathogen development and replication, such as the Flavivirus (6, 7, 14).

Table: Benefits of Vitamin C

Interferon	Supports production of interferon	Interferon establishes protective cellular defenses
Phagocytes	Enhances function of phagocytes	Phagocytes are white blood cells that envelop pathogens
Prostaglandin Formation	Enhances prostaglandin formation	Prostaglandins are hormone-like compounds that regulate T-lymphocyte function
T-lymphocyte	Inhibits various forms of T-	Helps keep T-lymphocyte alive

	lymphocyte death and enhance production	and enhances production
B-lymphocyte	Enhances B-lymphocyte production	B-lymphocytes are white blood cells that make antibodies

Flavivirus

Flaviviruses are similar to one another in several ways: common size (40-65 nm), nucleic acid (positive-sense, single-stranded RNA, 10,000-11,000 bases), and appearance under an electron microscope. Viruses within this genus are enveloped, with icosahedral and spherical geometries (7, 14). The diameter is approximately 50 nm and genomes are around 10-11kb in length with linear positive-sense RNA (7, 14). Moreover, Flavivirus, like most other viruses are small infectious agents that replicate only inside living cells of other organisms. These viruses are able to infect many types of life forms, from animals, to microorganisms (7, 14, 21). Viruses with the inclusion of the Flavivirus family are able to be found in almost every ecosystem on Earth.

If a virus is not inside an infected cell, they are able to exist in the form of independent particles (14). These viral particles, also identified as virons, are made up of two to three parts: (I) the genetic material derived from RNA or DNA, long molecules carrying genetic information, (II) a protein coat also known as the capsid, which protects the genetic material, and (III) an envelope of lipids that covers the protein coat when the particles are outside a cell (14, 21). The shapes of these particles vary from icosahedral to helical in some cases and are dependent on species. Most virons are about one one-hundredth the size of an average bacterium (7).

The origins of viruses, and specifically the Flavivirus, with regards to its evolutionary history of its life are still unclear to date. It is believed that some of the viruses may have evolved from plasmids or pieces of DNA that can travel between cells while others may have evolved from bacteria (7). With regards to evolution, viruses are important in the sense that through time it may create a horizontal gene transfer; which has been known to increase genetic diversity in hosts (20). Viruses are considered by some scholars to be a life form, for the reason of its ability to carry genetic material, evolve, and reproduce; however, viruses, including the Flavivirus lack important characteristics, such as cell structure. Having cell structure is a common characteristic of living organisms (20, 21).

There are different opinions on whether viruses are actually life forms or simply organic structures that interact with living organisms. In many instances, viruses have been described as organisms at the edge of life, because they have the capability of creating copies of themselves, similar to living organisms (10, 13, 17). However, viruses with the inclusion of Flaviviruses do not have their own metabolism. They require a host cell to replicate and make new products; therefore, cannot naturally reproduce outside of a host cell, a characteristic that most living organism's exhibit (20, 21).

In addition to this, viral populations do not develop and grow through cell division, because they are considered to be a-cellular (10, 13). Conversely, they use the metabolism of the host cell to create multiple copies of themselves, and subsequently assemble in the cell itself (10, 13). Nevertheless, if these viruses are not considered to be life forms, then at the very least, viruses possess some qualities that may characterize themselves as being dangerous, pathogenic organisms that are at the edge of life.

The body's initial defensive capability against viruses, such as the Flavivirus includes the immune system. This system includes cells and other mechanisms that defend against pathogens from infection (11, 12, 19). This means that the immune system should be able to recognize, and respond to pathogens in a generic way; however, unlike an adaptive immune system, that evolves

and changes over time to account for newer and more infectious pathogens, the human innate immune system may not confer long-lasting or protective immunity to the host (20, 21).

The majority of the viruses in the Flavivirus genus are considered to be arboviruses or viruses transmitted through mosquito, tick, or other arthropod bites. Infections from these viruses are typically identical with regards to the human hosts being a dead end for its life cycle; however, this isn't the case for yellow fever, dengue, and Zika viruses (4, 5). These viruses require mosquito vectors and can be intermittently transmitted between host and vector. Other viral transmission routes for the Flavivirus includes blood transfusion, exchange of bodily fluids, childbirth and through consumption of unpasteurized milk products (7, 8).

Flaviviruses are considered to be 5' capped positive-stranded RNA viruses. These viruses replicate their genomes within endoplasmic reticulum-derived vesicles (7, 8). Research has shown that Flaviviruses induce oxidative stress late in infection and that there is a symbiotic relationship with the pathogen, the host and in addition to this relationship, the oxidative stress acts as a benefactor for the pathogen (15, 16, 23). To validate this argument, research has shown that antioxidant treatment of the Flavivirus *in vitro* reduced virus production, viral positive-to-negative strand RNA ratio, and resulted the accumulation of uncapped positive-sense viral RNAs (7, 8, 23).

Moreover, treatment of the NS5 RNA capping enzyme in vitro with oxidizing agents enhanced guanylyltransferase activity (function of NS5 RNA capping enzyme) (13, 14, 23). This indicates that the guanylyltransferase function of the Flavivirus is activated by oxidative conditions, and adversely affected by antioxidant conditions (vitamin c). These findings suggest that RNA viruses, such as the Flavivirus may utilize oxidative stress during infection to help temporally control genome RNA capping and replication (23).

Oxidative Stress

Oxidative stress is comparatively the reflection of an imbalance between manifestation of reactive oxygen and the biological systems ability to readily detoxify (1, 22). Instabilities in the redox state of cells can create toxic effects through the creation of free radicals that may damage cells, proteins, lipids, and DNA (18). Flaviviruses have been known to induce oxidative stress in infected cells both in vitro and in vivo (23).

However, until recently, the induction of oxidative stress has only been theorized as simply a byproduct of the infection. Several recent studies have demonstrated the opposite, that modulating oxidative stress can alter Flavivirus replication (23). This means that treatment of infected cells with antioxidants, such as vitamin c could potentially yield promising results. Based on prior research, if oxidative stress positively affects viral RNA replication, then conversely antioxidant treatment can impair viral RNA replication and alter the amount of capped RNA present in cells (Original).

Antioxidant

Antioxidants are considered to be molecules that inhibit the oxidation of other molecules. In other terms, oxidation is a chemical reaction that can create free radicals, causing a chain reaction that has the potential to damage cells (15, 16). Various forms of antioxidants, such as vitamin c or thiols, terminate these chain reactions. Furthermore, in order to balance a state of oxidation, humans maintain a complex system of overlapping antioxidants, such as enzymes that are produced internally. In support of this process, dietary antioxidants, such as vitamin a, and c may be readily available after consumption and needed after incidences of oxidative stress (15, 16).

Antioxidants are classified into two different categories, depending on their solubility in water (hydrophilic) or in lipid (lipophilic). With regards to vitamin c, it is considered to be water soluble and an antioxidant with readily available electrons to be donated (1, 3, 22). With regards to water-soluble antioxidants, these antioxidants react with oxidants in the blood plasma and cell cytosol, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds are capable of being synthesized in the human body or obtained from a proper diet. The difference between various types of antioxidants may represent itself at a wide range of concentrations in bodily fluids and tissues (1, 22). For example, glutathione is mostly present within cells, while others, such as uric acid and vitamin c are more evenly distributed throughout the body (1, 3).

The importance and mechanisms behind the effect of different antioxidants within the human body is a complex question where various metabolites and enzyme systems have an interdependent effect on one another (22). The action of antioxidant may have an effect on other antioxidants within the system. Moreover, the amount of protection provided to the human body by one particular antioxidant, such as vitamin c is dependent on its concentration, and its reactivity towards a reactive oxygen species (22).

In addition, some compounds have the ability to contribute to the antioxidant defense mechanism by chelating transition metals and preventing them from catalyzing it in subsequently hindering the production of free radicals in the cell (22). This mechanism itself is antioxidant in nature along with the ability to sequester iron, and Zink; commonly referred to as antioxidant nutrients despite its lack of antioxidant capability on its own (22). These chemical elements are not considered antioxidants themselves but rather are required for the activity of some antioxidant enzymes (22).

Discussion

In order to truly understand the effect of vitamin c on the Flavivirus, this review has attempted to provide a basis of information in the effort to explain and review the characteristics and mechanism of vitamin c and its effect on the Flavivirus. This basis include a summarization of key components concerning the theory of the antioxidant effect on viral RNA replication, or in other words, the viricide effect of vitamin c. These components included oxidative stress reduction through the release of available electrons from vitamin c supplementation, and temporally control of genome RNA capping and replication by the reduction of oxidative stress (23). Following the illustration of the basis behind vitamin c and its antioxidant effect, this paper also attempted to incorporate a more in-depth understanding the Flavivirus genus, how the virus replicates and survives in host cells, and how it deals with the interaction between antioxidants and a supported immune system through vitamin c supplementation (23).

With regards to the interaction between antioxidants, ascorbic acid and the Flavivirus, previous research and studies have shown promising results both in vivo and in vitro with regards to vitamin c supplementation and its introduction to infectious diseases, such as the Dengue virus and West-Nile virus. In reference to Dr. Thomas E. Levy, *Vitamin C, Infectious Diseases, and Toxins: Curing the Incurable*, his literary work reveals actual accounts of success rates with vitamin c supplementation with hosts infected with the Flavivirus. More specifically, his studies have shown a promising success rates concerning 100% recover with over a half of dozen cases of dengue fever and West Nile virus, after ascorbic acid administration intravenously and or oral vitamin c supplementation.

Conclusion

The theory behind the actual effect of vitamin c on the Flavivirus is still new and for the most part relative to at least two variables, which includes the vitamin c antioxidant effect in the human body, the antioxidant effect on the Flavivirus and oxidative stress and ascorbic acid's ability to support the immune system and its functions in neutralizing the Flavivirus. Research has shown that vitamin c may in fact have the ability to impair Flavivirus viral RNA replication and alter the amount of capped RNA present in cells. In laments terms, enough vitamin c supplementation may hinder the Flavivirus from replication, spreading throughout the body, and consequently allow antibodies to defend against further infection.

In relation to the factors that affect the relationship between ascorbic acid and the Flavivirus, vitamin c has been known to (I) positively affect interferon production, (II) phagocytes function, (III) T and B-lymphocyte production, and (IV) support the immune system. In relation to the relationship, vitamin c at the very least has an antagonist effect on the Flavivirus. This effect positively supports the immune system, and may prevent viral proliferation throughout the human body. Ultimately, this review concerning "The Effect of Vitamin C on the Flavivirus" had to be extensive, and more research should be done in order to gain a better understanding on the mechanism behind Dr. Levy's positive results with treating patient's positive with Flavivirus. More research should be conducted on this topic in order to develop and understand more effective treatments or supplementations in response to the Flavivirus.

References

1. Aydogan, M., & Korkmaz, A. (2011). *Does vitamin C act as an antioxidant or prooxidant?* New York: Nova Biomedical Books.
2. Benedetti, S., Nuvoli, B., Catalani, S., & Galati, R. (2015). Reactive oxygen species a double-edged sword for mesothelioma. *Oncotarget*, *6*(19), 16848-16865. doi:10.18632/oncotarget.4253
3. Cass, M. D., & English, J. (2013). *User's Guide to Vitamin C: Learn What You Need to Know about How Vitamin C Can Improve Your Total Health*. Laguna Beach: Basic Health Publications.
4. Centers for Disease Control and Prevention (U.S.). (2016). *Zika and sex: information for pregnant women living in areas with Zika*.

5. Das, B. P. (2012). *Mosquito vectors of Japanese encephalitis virus from Northern India: Role of BPD hop cage method*. New Delhi: Springer India.

6. Devreese, L., & Gilbert, C. C. (2015). Phylogenetic relationships within the C ercocebus- M andrillus clade as indicated by craniodental morphology: Implications for evolutionary biogeography. *Am. J. Phys. Anthropol*, *158*(2), 227-241. doi:10.1002/ajpa.22780

7. Diosa-Toro, M., Urcuqui-Inchima, S., & Smit, J. M. (2013). Arthropod-Borne Flaviviruses and RNA Interference. *Advances in Virus Research*, 91-111. doi:10.1016/b978-0-12-408116-1.00004-5

8. Flaviviridae| Viral Hemorrhagic Fevers (VHFs) | CDC. (2014). Retrieved from http://www.cdc.gov/vhf/virus-families/flaviviridae.html

9. Galvin, J. E. (2015). Medical Foods and Dietary Approaches in Cognitive Decline, Mild Cognitive Impairment, and Dementia. *Diet and Nutrition in Dementia and Cognitive Decline*, 343-356. doi:10.1016/b978-0-12-407824-6.00031-8

10. Gebhard, L. G., Filomatori, C. V., & Gamarnik, A. V. (2011). Functional RNA Elements in the Dengue Virus Genome. *Viruses*, *3*(12), 1739-1756. doi:10.3390/v3091739

11. Gosai, P. A. (2012). *Ascorbate concentration in lung and heart tissue of mice after septic insult and parenteral administration of Ascorbic Acid and Dehydroascorbic Acid*.

12. Guan, Y., Tapping, R. I., Tapping, R. I., Slauch, J. M., Orlean, P. A., & Whitaker, R. J. (2011). *Innate immune recognition by human toll-like receptor 10 and other members of the toll-like receptor 2 subfamily*. Urbana, IL: University of Illinois.

13. Gullberg, R., Steel, J., Moon, S., Soltani, E., & Geiss, B. (2015, January 15). Oxidative Stress Influences Positive Strand RNA Virus Genome Synthesis and Capping. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4332586/

14. In Acton, Q. A. (2012). *Flavivirus: Advances in research and treatment: ScholarlyBrief*.

15. In Dziubla, T., & In Butterfield, D. A. (2016). *Oxidative stress and biomaterials*.

16. In Jakob, U., & In Reichmann, D. (2013). *Oxidative stress and redox regulation*.

17. Lim, S., & Lescar, J. (2013). Dengue virus RNA dependent RNA polymerase with residues from the NS5 linker region. doi:10.2210/pdb4c11/pdb

18. Pratviel, G. (2011). Oxidative DNA Damage Mediated by Transition Metal Ions and Their Complexes. *Metal Ions in Life Sciences*, 201-216. doi:10.1007/978-94-007-2172-2_7

19. Ross, A. C. (2014). *Modern nutrition in health and disease*. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

20. S mall f lavivirus RNA (sfRNA). (2015). *The Dictionary of Genomics, Transcriptomics and Proteomics*, 1-1. doi:10.1002/9783527678679.dg12126

21. South Carolina. (2015). *2014 Flavivirus virus cases*.

22. Uzilday, B., Ozgur, R., Sekmen, A. H., & Turkan, I. (2015). Redox Regulation and Antioxidant Defence During Abiotic Stress: What Have We Learned from Arabidopsis and Its Relatives? *Reactive Oxygen Species and Oxidative Damage in Plants Under Stress*, 83-113. doi:10.1007/978-3-319-20421-5_4

23. Gullberg, R. C., Jordan Steel, J., Moon, S. L., Soltani, E., & Geiss, B. J. (2015). Oxidative stress influences positive strand RNA virus genome synthesis and capping. Virology, 475, 219-229. doi:10.1016/j.virol.2014.10.037

24. Levy, T. E. (2002). *Curing the incurable: Vitamin C, infectious diseases, and toxins*. Henderson, NV: Livon Books.